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Cord blood serum creatine kinase isoenzymes with placental dysfunction

Waldemar Niklinski[1], Zdzislaw Palynyczko[3], Michal Jozwik[2], and Adam Sledziewski[3]

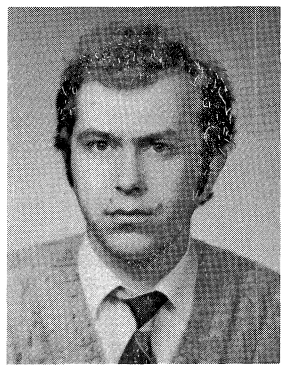
[1]Clinic of Gynecology, Institute of Obstetrics and Gynecology, Medical Academy, Bialystok, Poland
[2]Department of Perinatology, Institute of Mother and Child, Bialystok, Poland
[3]Laboratory Department, Railways' Hospital, Warsaw, Poland

1 Introduction

Although a number of theories have been proposed, the pathogenesis of perinatal brain damage is still not fully understood. Recently it has been suggested that perinatal asphyxia is not followed by neurogenic abnormalities in the infant unless there is preexisting chronic fetal distress [10]. Hypertensive disease, prolonged pregnancy or intrauterine fetal growth retardation are examples of such conditions. Precise diagnosis and evaluation of brain injury remain two of the most difficult problems in the perinatal period. Enzymatic determinations appear promising because damaged cells may release by-products into body fluids from which enzymic activities can be detected. Elevated levels of brain type creatine kinase isoenzyme (CK-BB) have been demonstrated in serum after, either ischemic or traumatic, brain injury in adults [12, 15]. Other authors have reported the rise of CK-BB levels in instances of asphyxic insult [6, 7], intraventricular hemorrhage [19, 20] and perinatal brain damage in neonates [9].

The purpose of this study is to evaluate the levels of CK isoenzymes in umbilical cord sera from newborns who experienced chronic fetal distress caused by placental dysfunction characterized by diminished level of human placental lactogen (HPL) and cystine aminopeptidase (CAP) activity. We evaluated the clinical state of newborns using the 5 minutes Apgar score.

Curriculum vitae



WALDEMAR NIKLINSKI, *M. D. was born in Poland in 1956. He studied medicine at the Medical Academy in Bialystok, and he was graduated in 1981. The Doctor of Medicine was awarded in 1985. He trained on gynecology and obstetrics at the Clinic of Gynecology and Obstetrics at the Medical Academy in Bialystok. His interests include fetal asphyxia, brain damage and the use of ultrasound in obstetrics and gynecology.*

2 Material and methods

Our investigations were performed in a group of 57 newborns delivered after 37 weeks of pregnancy. Gestational age was estimated using the first day of the last menstrual period and by ultrasonic evaluation. For the study of creatine kinase activity in umbilical cord sera two groups of newborns were selected according to placental function. The first group consisted of infants born of mothers with normal placental function and the second group included cases with clinically suspected placental dysfunction. Two criteria of placental function were used, i. e. HPL level and CAP activity as measured in blood sera of pregnant women. We examined CAP activity in blood sera by the method of VAN OUDHEUSDEN [22]

modified by JOZWIK et al. [11] and the HPL level was determined using a commercial kit (Organon Teknika HPL-Nosticon, Elisa system). We have used the last obtained values prior to the onset of labor. In our studies we accepted the normal lower limit of HPL as higher than 5 mg/l (as determined and recommended by Organon Teknika, Holland) and a CAP activity greater than 70 U/l (as determined in our laboratory). Pregnant patients with an HPL level and a CAP activity above normal limits were included into the group of patients with normal function of the placenta (25 cases, figure 1). In the remaining cases, placental dysfunction has been suspected.

In an attempt to emphasize placental malfunction and to make our results relevant and reliable, the term of placental dysfunction was used in cases with a simultaneously reduced HPL level and decreased CAP activity (21 cases, figure 1). Newborns blood was drawn from the umbilical cord at the time of delivery. All blood samples were centrifuged and sera analyzed immediately or stored at −20 °C. Isoenzymes of creatine kinase were separated by ion exchange column chromatography as described by BONDAR et al. [5]. Enzymatic activity was measured using kits supplied by Boehringer-Mannheim (Monotest® CK-NAC aktiviert). Newborns were considered asphyxiated with a 5 minutes Apgar score of 7 or less. Of 57 newly born infants, 15 had features of perinatal asphyxia.

Since enzymatic values were not normally distributed, statistical comparisons were made using a non-parametric test (S-Kendall's test) [1].

3 Results

Total CK activity in cord sera ranged from 40 to 400 U/l. Isoenzymic distribution for skeletal muscle type CK (CK-MM) ranged from 17 to 306 U/l, for heart muscle type CK (CK-MB) from 1.8 to 30 U/l and for CK-BB from 4 to 335 U/l. We were not able to find significant differences in both total and isoenzymic (CK-MM, CK-MB) activities of creatine kinase in infants born of mothers with either normal or impaired placental function. There were no essential differences in total CK, CK-MM and CK-MB in either normal or asphyxiated newborns. Our studies demonstrated a significant rise of CK-BB activity in cord sera of newborns delivered from pregnancies with placental dysfunction (figure 2) as well as in cases of asphyxiated infants (figure 3).

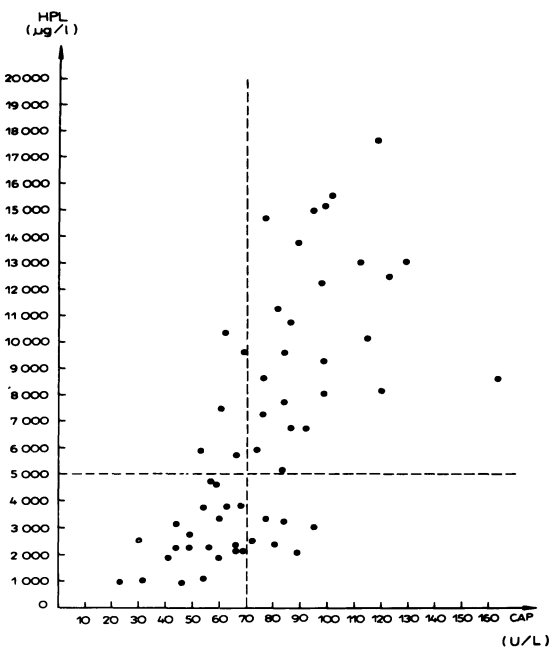


Figure 1. CAP activity and HPL level in sera of examined pregnant women.

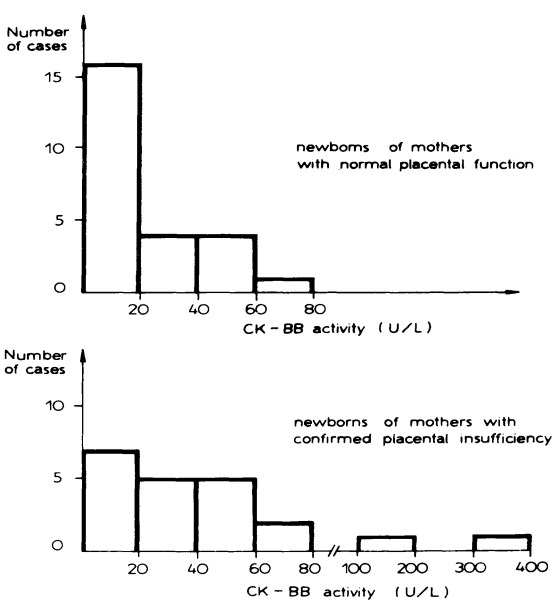


Figure 2. Histograms of brain type creatine kinase isoenzyme (CK-BB) activity in umbilical cord serum of newborns in relation to placental function. S-Kendall's test significant $p < 0.05$.

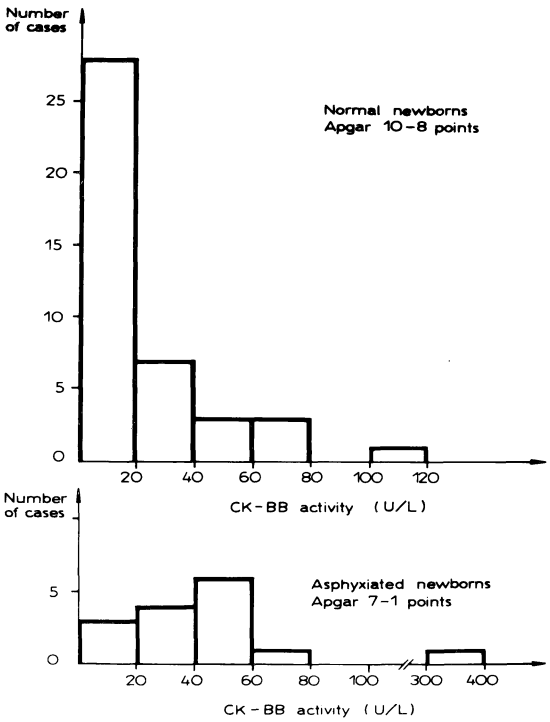


Figure 3. Histograms of brain type creatine kinase isoenzyme (CK-BB) activity in umbilical cord serum of newborns evaluated by 5 minutes Apgar score. S-Kendall's test significant $p < 0.01$.

4 Discussion

We found that the main source of total CK enzymatic activity in cord blood was derived from the CK-MM fraction. A few of our cases demonstrated a higher activity of CK-BB isoenzyme. During intrauterine hypoxia the fetus is able to use well known mechanisms of integrated cardiovascular response [17]. Since there is impaired skeletal muscle blood flow during hypoxia, in cases of placental dysfunction and/or fetal asphyxia, we might expect increased release of CK-MM from skeletal muscles as well as a rise in total CK activity. In spite of this we were unable to find a relationship between placental dysfunction or fetal asphyxia and total CK or CK-MM activities in cord blood. One possible explanation is that skeletal muscle is scarcely vulnerable to an hypoxic state. We realize that any intramuscular drug injection may result in a CK increase in blood serum [16]. The second hypothesis is compatible with metabolic impairment of skeletal muscles during fetal hypoxia resulting in CK release to lymphatic space. LINDENA et al. [14] demonstrated six times higher CK activity in lymph as compared to serum. Lymph flow is related to muscle activity. Ischemia is a factor causing a fall in lymph flow. Other authors have observed the increase of CK-MM and total CK activity in blood sera as early as several hours after normal delivery [2, 7]. It may be correlated with elevated activity and rise in perfusion of muscles of the newborn. BODENSTEINER [4] found markedly higher levels of total CK within 24 hours following normal vaginal delivery as opposed to cases delivered by cesarean section, suggesting that the trauma related to natural birth may be responsible for that phenomenon. We found that newborns delivered with in utero placental dysfunction and infants with low Apgar scores have significantly increased CK-BB activity in cord blood. To date it is not known if this elevated CK-BB activity could be considered as a possible indicator of cerebral injury. During chronic fetal distress in pregnancies with placental dysfunction, the fetus utilizes several compensatory mechanisms, but its ability to survive is handicapped after an acute episode of hypoxia as compared to cases of normal uncomplicated pregnancy [3]. Thus, in gestations affected by placental insufficiency, brain injury is more frequent. Increased CK-BB activity in this group of newborns may reflect this situation. Others have confirmed that severe asphyxia resulted in an increase of CK-BB activity in cord blood [6, 7]. In addition, infants with ominous fetal heart rate patterns have higher CK-BB activity [8]. There are several possible sources for CK-BB activity in cord blood. CK-BB activity in cord blood could be a result of enzyme loss from placental tissue. Enzymic activity from placenta was markedly increased in mild and severe pre-eclampsia [18]. The human placenta contains only small amounts of both, total CK and CK-BB; thus its activities should be small or negligible [6]. CK-BB can also be liberated from the uterus where it is found in considerable amounts [21]. Umbilical artery and vein contain high CK-BB activity [13]. BB isoenzyme can come from fetal lungs and gastrointestinal tract [21]. In spite of this fact, CUESTAS [7] could not demonstrate an elevation of this enzyme in neonates with purely pulmonary or gastrointestinal tract disease. Although different organs do contain CK-BB, the brain is most likely to represent the source of elevated CK-BB activity found in cord blood in cases of placental dysfunction.

Summary

It has been suggested that perinatal asphyxia is not generally followed by neurological impairment unless there is preexisting chronic fetal distress. In cases of brain damage one can observe elevated levels of CK-BB. The purpose of our study was to evaluate CK isoenzymes in umbilical cord blood sera of newborns affected by chronic fetal distress. Fetal distress reflected by placental dysfunction was characterized by a diminished HPL level and decreased activity of CAP. We estimated CK isoenzymes with the use of DEAE-sepharose CL-6B column chromatography. Total CK activity was measured using kits supplied by Boehringer-Mannheim (Monotest® CK-NAC aktiviert). The clinical state of examined newborns was estimated. Investigations were carried out in the group of 57 infants delivered after 37 weeks of gestation. Total CK activity in cord sera ranged from 40 to 400 U/l. Our results showed a significant rise of CK-BB activity in cord sera of newborns delivered from pregnancies with placental dysfunction (figure 2) as well as in cases of asphyxiated infants (figure 3). We were unable to demonstrate differences in total CK, CK-MM and CK-MB activities in all examined groups of newborns. Other authors have confirmed that severe asphyxia results in increase in CK-BB activity in cord blood. Infants with ominous fetal heart rate patterns have higher CK-BB activity. There are several possible sources for CK-BB activity in umbilical cord blood sera, i. e. fetal brain, lung, gastrointestinal tract, placenta and uterus. It appears that the brain is most likely the source of elevated CK-BB activity found in cord blood in cases of placental dysfunction.

Keywords: Brain damage, cord blood, creatine kinase isoenzymes, examination, fetal asphyxia, placental dysfunction.

Zusammenfassung

Die Isoenzyme der Serum-Kreatinin-Kinase im Nabelschnurblut bei ungenügender Plazenta-Funktion

Es wurde vermutet, daß die perinatale Asphyxie nicht unbedingt von neurologischen Ausfallerscheinungen gefolgt sein muß, gesichert scheint dies nur bei vorherbestehenden, chronischen fetalen Dystrophien. In den Fällen, bei denen ein Gehirnschaden auftrat, konnte ein erhöhter CK-BB-Spiegel gemessen werden. Das Ziel unserer Studie war die Auswertung der CK-Isoenzyme im Serum aus Nabelschnurblut Neugeborener, die von chronischer fetaler Dystrophie in utero betroffen waren. Fetale Dystrophie, angezeigt durch plazentare Dysfunktion, war durch verminderte HPL-Spiegel und verminderte CAP-Aktivität charakterisiert. Wir schätzten die CK-Isoenzyme mit Hilfe der DEAE-Sepharose CL-6B Säulenchromatographie, die gesamte CK-Aktivität wurde mit Boehringer-Mannheim (Monotest® CK-NAC aktiviert) gemessen. Zusätzlich wurde der klinische Status der untersuchten Neugeborenen bestimmt. Die Untersuchungen wurden in einer Gruppe von 57 Neugeborenen durchgeführt, die in der 37. Gestationswoche geboren wurden. Die gesamte CK-Aktivität im Nabelserum lag zwischen 40 und 400 U/l. Unsere Untersuchungen zeigten einen deutlichen Anstieg der CK-BB-Aktivität in den Nabelschnursera von Neugeborenen aus Schwangerschaften mit einer plazentaren Unterfunktion (Abb. 2) wie auch in den Fällen von fetaler Asphyxie (Abb. 3). Wir konnten in allen untersuchten Gruppen von Neugeborenen keine Unterschiede in den Gesamt-CK-, den CK-MM- und den CK-MB-Aktivitäten messen. Andere Autoren bestätigten, daß ausgeprägte Asphyxie einen Anstieg der CK-BB-Aktivität im Nabelschnurblut zur Folge hat. Weiterhin zeigen Neugeborene mit krankhaften fetalen Herzfrequenzmustern eine erhöhte CK-BB-Aktivität. Als Ursprung für die CK-BB-Aktivität im Serum aus Nabelschnurblut kommen mehrere Möglichkeiten in Betracht, z. B. das fetale Gehirn, die Lunge, der Gastrointestinaltrakt, die Plazenta und der Uterus. Jedoch scheint bei plazentarer Unterfunktion der Ursprung für die erhöhte CK-BB-Aktivität im Gehirn zu liegen.

Schlüsselwörter: Fetale Asphyxie, Gehirnschaden, Isoenzyme der Kreatininkinase, Nabelschnurblutuntersuchung, plazentare Unterfunktion.

Résumé

Isoenzymes de la créatine kinase au sang du cordon en cas de dysfonction placentaire

On a suggéré que l'asphyxie périnatale n'entraine pas de déficit neurologique sauf s'il existe une souffrance fœtale chronique antérieure. En cas de lésions cérébrales, on a pu observer une élévation des taux de CK-BB. Le but de notre étude a été d'évaluer les isoenzymes de la CK au sang du cordon ombilical chez les nouveaux-nés ayant présenté une souffrance fœtale chronique in utero. La souffrance fœtale manifestée par une dysfonction placentaire est caractérisée par une diminution de l'HPL et une diminution de l'activité de la CAP. Nous avons dosé les isoenzymes de la CK en utilisant une chromatographie sur colonne de DEAE-sepharose CL-6B, l'activité CK totale a été mesurée en utilisant des kits fournis par Boehringer-Mannheim (Monotest® CK-NAC aktiviert). De plus, on a estimé l'état clinique des nouveaux-nés étudiés. Ces explorations ont été effectuées sur un groupe de 57 enfants nés après 37 semaines de gestation. L'activité CK totale varie de 40 à 400 U/l. Nos résultats

montrent une élévation de l'activité CK-BB au sang du cordon chez les nouveaux-nés nés après une grossesse ayant présenté une dysfonction placentaire (figure 2) mais aussi chez les enfants asphyxiques (figure 3). Nous ne sommes pas capables de démontrer des différences de la CK totale, et des activités CK-MM et CK-MB entre les groupes de nouveaux-nés examinés. D'autres auteurs ont confirmé que l'asphyxie sévère entraîne une augmentation de l'activité CK-BB au sang du cordon. De plus, les enfants avec un aspect inquiétant du rythme cardiaque fœtal ont une activité CK-BB plus élevée. Il y a plusieurs sources possibles pour l'activité CK-BB au sang du cordon, à savoir le cerveau, les poumons, le tractus gastro-intestinal du fœtus, le placenta et l'utérus. Cependant, il semble que le cerveau soit la source la plus probable de l'activité CK-BB élevée trouvée au sang du cordon en cas de dysfonction placentaire.

Mots-clés: Asphyxie fœtale, dysfonction placentaire, examen au sang du cordon, isoenzymes de la créatine kinase, lésion cérébrale.

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Waldemar Niklinski, M. D.
Clinic of Gynecology
Institute of Obstet. Gynec.
Medical Academy
M. C. Sklodowskiej 24 a
15–276 Bialystok, Poland